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Baeomyces lotiformis sp. nov. from China

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Abstract—A new species of lichenized fungus, *Baeomyces lotiformis*, is described and illustrated. It is distinguished by its short podetia and wide apothecium discs, and its affinity was confirmed by ITS sequence analysis.

Key words—*Ascomycota*, *Baeomycetaceae*, chemistry, molecular systematics, morphology

Introduction

The worldwide lichen genus *Baeomyces* Pers. is known from eight crustose to squamulose species with dark red to brownish stiped apothecia containing stictic acid as a major secondary substance (Asahina 1943, Upreti 1985, Gierl & Kalb 1993, Ihlen 1997, Aptroot & Seaward 1999, Kirk et al. 2008). In Yunnan we encountered an undescribed species that showed affinities with this genus but deviated conspicuously by its confluent lotus-like apothecia on very short stipes. Here we present our analysis of the morphology, anatomy, chemistry, and phylogeny of this species to clarify its taxonomy.

Materials & methods

The material used in this study was collected in 2012 in Yunnan province, China. The collections examined are preserved in the Lichen Section of the Botanical Herbarium,

Table 1. nrDNA ITS sequences used in the phylogenetic analysis

Species	Locality	GenBank No.
Ainoa mooreana	Sweden	KJ462262
	Japan	KJ462263
Anamylopsora pulcherrima	Buryatia Republic, Russia	KR017064
	—	AF274089
Baeomyces lotiformis	**Yunnan, China**	**KT597690**
Baeomyces placophyllus	China	DQ001274
	Xizang, China	**KT601493**
Baeomyces rufus	**Yunnan, China**	**KT601494**
	France	AF448458
Parainoa subconcolor	Yunnan, China	KR017133
Phyllobaeis crustacea	Hainan, China	KC414617
	Hainan, China	KC414618
Phyllobaeis imbricata	Ecuador	KC414619
	—	HQ650635
Placopsis contortuplicata	Antarctica	DQ534479
	Antarctica	**KT601492**
Placopsis macrophthalma	Kerguelen	AY212820
Trapelia placodioides	—	KT695380
Trapelia thieleana	—	KU672616
Trapeliopsis flexuosa	—	HQ650634
Trapeliopsis glaucolepidea	—	AY600082

*New sequences are marked in **bold** font; missing data are indicated with "—".

Shandong Normal University, Jinan, China (SDNU). A Zeiss Axioskop 2 plus compound microscope and Motic SMZ-168 dissecting microscope were used to study the anatomy and morphology. A 10% KOH solution, a 5% NaOCl bleaching solution, concentrated alcoholic *p*-phenylenediamine (PD), Lugol's Iodine solution, and thin-layer chromatography (TLC) (Culberson & Kristinsson 1970, Culberson 1972, White & James 1985) were used to detect lichen substances.

DNA extraction, PCR amplification, and sequencing

Total DNA was extracted using a modified CTAB method (Cao et al. 2013). PCR amplifications were performed using a Biometra T-Gradient thermal cycler. The nrDNA ITS region was amplified by the primer pair ITS5 and ITS4 (White et al. 1990). The 50-µl reaction volume comprised 1 µl total DNA, 2 µl of each primer (10 µM), 1 µl Taq polymerase (rTaq DNA Polymerase, 5 U/µl), 4 µl dNTP (2.5 mM each), 5 µl amplification buffer (10×, 25 mM $MgCl_2$ contained), and 35 µl ddH_2O. Cycling parameters included initial denaturation at 95°C for 5 min, 30 cycles of denaturation at 94°C for 40 s, annealing at 52°C for 40 s, extension at 72°C for 2 min, and a final extension at 72°C for 10 min. A negative control, without DNA template, was prepared in every amplification series so as to minimize the possibility of contamination.

PCR products were purified using a Biocolor gel purification kit (BioScience & Technology Co. Ltd.) and sequenced using an ABI 3730 XL DNA Sequencer.

Four nrDNA ITS sequences, including one from the new species, were obtained by the authors, and another 17 nrDNA ITS sequences representing six species were downloaded from GenBank (Table 1). Three sequences from *Placopsis* (*Trapeliaceae*), two from *Trapelia* (*Trapeliaceae*) and two from *Trapeliopsis* (*Trapeliaceae*) were selected as outgroup.

Phylogenetic analysis

The sequences were aligned using ClustalW 1.6 (Thompson et al. 1994). The phylogenetic analysis was executed with software Mega 7 (Kumar et al. 2016). The Kimura-2-parameter was selected as the nucleotide substitution model, and gaps or missing data were set as pairwise deletion. The neighbor-joining (NJ) method was used in constructing the phylogenetic tree and the reliability of the inferred tree was tested by 1000 bootstrap replications.

Result and discussion

The NJ-tree (Fig. 1) of the ITS rDNA sequences clearly supports the genera *Baeomyces*, *Phyllobaeis*, *Parainoa*, *Ainoa*, and *Anamylopsora* in *Baeomycetaceae* as sister branches. The bootstrap values were 98% for *Baeomyces* and 97% for *Phyllobaeis*. *Baeomyces lotiformis* clustered within the *Baeomyces* branch, but was clearly separated from *B. placophyllus* and *B. rufus*.

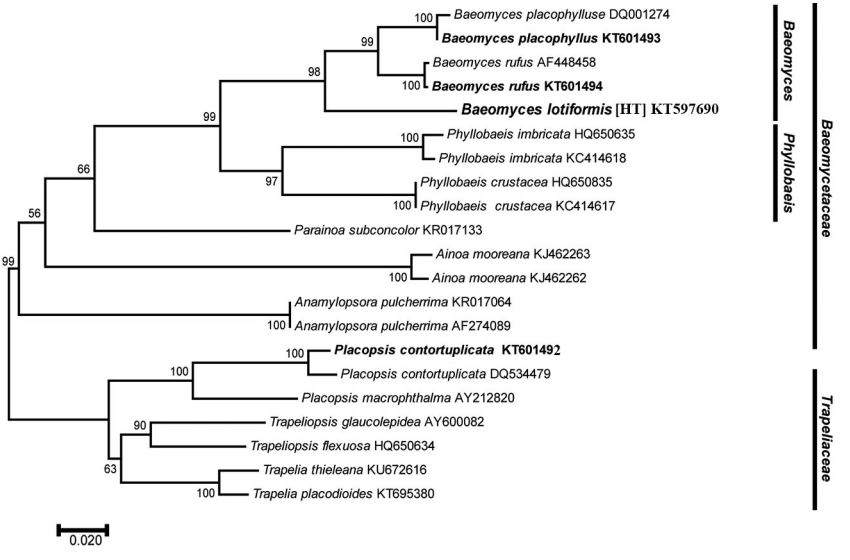

Fig. 1. Neighbor-joining tree of the nrDNA ITS region sequences. Nucleotide: K2 model, gaps or missing data were partial deletion, bootstrap = 1000. Genetic distance scale = 0.02. Numbers at nodes present the bootstrap support value (numbers <50 not shown). New sequences are set in bold font; holotype sequence indicated by '[HT]'.

Taxonomy

Baeomyces lotiformis S.N. Cao, **sp. nov.** Fig. 2

MycoBank MB 819009

Differs from the other *Baeomyces* species by its confluent, lotus-like apothecia and crustose thallus.

Type: China, Yunnan: Jianchuan County, 26°23'N 100°49.8'E, alt. 2665 m, on soil, 25 Nov. 2012, H. Y. Wang yn013 (**Holotype**, SDNU 20160915; GenBank KT597690).

Etymology: Latin *lotiformis*, referring to the lotus-like apothecia. The Latin noun "lotus" has the compounding form "lot–"; and the suffix indicating a morphological similarity is "–formis".

Thallus crustose, gray green to ochre, tightly attached to the substrate, lacking a cortical layer, irregularly delimited; surface irregular with many small, mammillary warts, 0.2 mm in diameter; algae layer continuous, algal cells green, ovoid or ellipsoid, 9–11 × 10–13 µm.

Apothecia dark red to reddish brown, scattered over the thallus, when full-grown with very sinuous margins deeply dividing the disc, which is thus seemingly composed of many confluent discs aggregated into a lotus-like structure, about 2 mm in diameter, when young rounded and 0.5 mm in diameter; disc flattened with swollen margin; podetia very short, less than 1 mm, with nearly sessile apothecia, lacking algae; hymenium 162.5–175 µm thick, algae not observed within subhymenium and exciple, I–; paraphyses branched, not septate; asci long-clavate, 8-spored, with apex I–, 75–90 × 7.5 µm; ascospores oblong or fusiform, hyaline, simple, 8–10 × 5 µm (10 spores observed).

Chemistry—Spot tests: Thallus K+ yellow, C–. Specimen contains stictic acid (TLC).

Additional material examined: **China**, **Yunnan**: Mt. Ailao, 24°21′N 102°31.2′E, alt. 2300 m, on rock, 25 November 2010, H.Y. Wang yn076 (SDNU 20160916).

Comments: *Baeomyces lotiformis* is characterized by a crustose thallus and dark red to reddish brown apothecia with very short podetia, which show affinities with the well-known stiped crustose to squamulose lichen genera *Baeomyces*, *Dibaeis* Clements, and *Phyllobaeis* Kalb & Gierl. However, the new species differs chemically from *Dibaeis* and *Phyllobaeis:* stictic acid is the main substance, and baeomycesic and norstictic acid are absent. The morphological and chemical characters support inclusion of *B. lotiformis* in *Baeomyces*, but within this genus its confluent, lotus-like apothecia are unique.

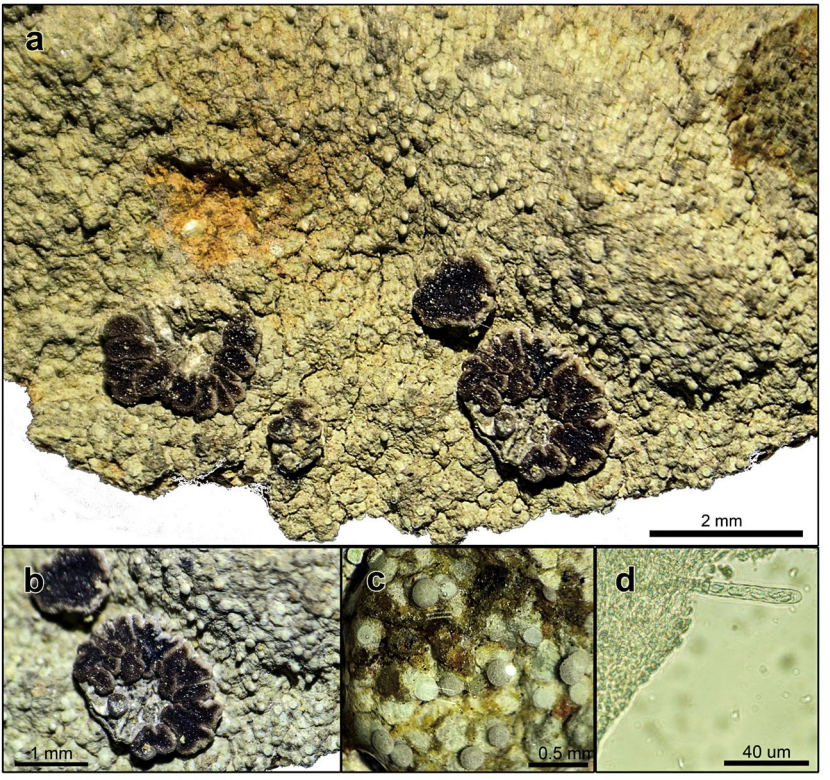

Fig. 2. *Baeomyces lotiformis* (holotype, SDNU 20160915). a, habit; b, apothecia; c, granular mammillary warts (in water); d, asci.

In view of its very short podetia, we compared *B. lotiformis* with the crustose lichen genera also currently included in *Baeomycetaceae: Ainoa* Lumbsch & I. Schmitt, *Anamylopsora* Timdal, and *Parainoa* Resl & T. Sprib. (Lücking et al. 2016). *Ainoa* differs by its dark-brown exciple, narrowly ellipsoid ascospores (16–32× 6–14 µm), and by the presence of gyrophoric acid restricted to apothecia and pycnidia (Brodo & Lendemer 2015). *Parainoa* is similar to *Ainoa* but differs in containing depsidones (like *Baeomyces*); however, *Parainoa* completely lacks a differentiated and extended hypothecial stalk, its hypothecium is yellowish, its thallus is creamish white, its ascomata may or may not contain gyrophoric acid, and it is characterized by simple, roundish apothecia (Resl et al. 2015). *Anamylopsora* is still more distinct by

its squamulose thallus, its black sessile apothecia, its subspherical ascospores, and the presence of alectorialic acid (Timdal 1991).

Our ITS rDNA sequence analysis clusters *B. lotiformis* with *B. rufus* and *B. placophyllus* on a well-supported branch and support *B. lotiformis* as a new species in the genus *Baeomyces*.

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